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(81) Bestimmungsstaaten *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

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Veröffentlicht:
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Zur Erklärung der Zweibuchstaben-Codes und der anderen Abkürzungen wird auf die Erklärungen ("Guidance Notes on Codes and Abbreviations") am Anfang jeder regulären Ausgabe der PCT-Gazette verwiesen.

(54) Title: DIAGNOSIS OF DISEASES ASSOCIATED WITH THE IMMUNE SYSTEM

(54) Bezeichnung: DIAGNOSE VON MIT DEM IMMUNSYSTEM ASSOZIIERTEN KRANKHEITEN

(57) Abstract: The invention relates to chemically modified genomic sequences of genes associated with the immune system, an oligonucleotide directed against said sequence and/or PNA oligomers for the detection of the methylation state of cytosine of genes associated with the immune system. The invention also relates to a method for determining genetic and/or epigenetic parameters of genes associated with the immune system.

(57) Zusammenfassung: Die vorliegende Erfindung betrifft die chemisch modifizierte genomische Sequenzen von mit dem Immunsystem assoziierten Genen, gegen die Sequenz gerichtete Oligonukleotide und/oder PNA-Oligomere zur Detektion des Cytosin-Methylierungszustandes von mit dem Immunsystem assoziierten Genen sowie ein Verfahren zur Ermittlung von genetischen und/oder epigenetischen Parametern von mit dem Immunsystem assoziierten Genen.

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Nucleic acid comprising fragment of chemically modified gene, useful for diagnosis and treatment of diseases associated with abnormal cytosine methylation

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Abstract (Basic): WO 200200928 A2

NOVELTY - A nucleic acid (I) comprising a segment of at least 18 bases of a chemical pretreated DNA of a gene (A) associated with the immune system, or its complement, where (A) is any one of 2420 sequences not given in the specification, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:

(1) oligomers (II) (oligonucleotides or peptide-nucleic acid (PNA) oligomers) for detecting the status of cytosine methylation in chemically treated DNA and comprising at least 9 nucleotides able to hybridize to chemically pretreated (A) or their complements;

(2) a set of at least two (II);

(3) preparing an array of (II) on a solid phase;

(4) arrays produced by method (3);

(5) DNA and/or PNA arrays, for analysis of diseases related to methylation status of genes, containing at least one (I);

(6) detecting genetic and/or epigenetic parameters for diagnosis and/or treatment of diseases (or predisposition) by analysis of cytosine methylation; and

(7) a kit containing bisulfite and (II).

ACTIVITY - Antiasthmatic; antiarteriosclerotic; antianemic; cytostatic; nootropic; neuroprotective; anti-HIV; anticonvulsant; ophthalmological; antirheumatic; antiarthritic; antidiabetic; antipsoriatic; antiinflammatory. No details of tests for any of these activities are given.

MECHANISM OF ACTION - None given in the source material.

USE - (I), and related oligomers (II) (or arrays of (II)), are useful for diagnosis, prognosis and/or treatment of immune system

disorders, particularly where associated with aberrant cytosine methylation of the specified genes, e.g. eye diseases ((diabetic) retinopathy, neovascular glaucoma or macular degeneration); arteriosclerosis; anemia; (pancreatic) cancer; acute myeloid leukemia; Alzheimer's disease; AIDS; epilepsy; neurofibromatosis; rheumatoid arthritis; psoriasis; and inflammatory/ulcerative bowel diseases.

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Technology Focus:

TECHNOLOGY FOCUS - BIOTECHNOLOGY - Preferred Gene: More than 300 (A) are tabulated, with their GenBank accession numbers.

Preferred Oligomer: These include at least one CpG dinucleotide, particularly in the middle third of the sequence. The set of at least 2 (II) particularly contains:

(i) (II) for determining the methylation status of all CpG dinucleotides from (A);

(ii) primer oligonucleotides for amplification of (A) or their complements (in this case the oligomers may be immobilized); or

(iii) at least 10 oligomers for detecting both cytosine methylation and/or single nucleotide polymorphisms in (A).

Preferred Array: (II) are arranged on a flat surface (of silicon, polystyrene, aluminum, steel, iron, copper, nickel, silver or gold) in a rectangular or hexagonal pattern.

Preferred Process: In method (6), a genomic DNA sample is treated, especially with bisulfite than alkali, to convert 5-unmethylated cytosines to uracil (or some other base with base-pairing properties different from those of cytosine) and fragments are amplified from the treated DNA using primer-(II). The amplicons, which include a detectable label, are then tested for hybridization to a set, or array, of probe-(II), and hybridized amplicons detected. At least 10 different fragments, of 100-2000 base pairs (bp), are amplified, in the same vessel, using a heat-stable polymerase and polymerase chain reaction for amplification. The amplicons are labeled with a fluorophore, radionuclide or a releasable molecular fragment of known mass, detectable by mass spectrometry (MS). Especially the amplicons, or their fragments (especially those with a single net charge for improved detection), are detected by MS, particularly using the matrix-assisted laser desorption/ionization or electrospray ionization techniques. The DNA being tested is isolated from e.g. cell lines, biopsies, blood, paraffin-embedded tissues etc.